"Potential of Algae in Bioremediation of Waste Water"

A synopsis of research work proposed to be carried out in pursuance of the requirement for the award of degree of Doctor of Philosophy in Botany (Microbiology)

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September, 2012

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INTRODUCTION

Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. Bioremediation is a cost effective and efficient method of decontamination that has become increasingly popular now-a- days to reduce environmental pollution. In urban and semi-urban colonies, sewage disposal has become an ecological problem (Moore, 1998). The effluents from residential and industrial discharge constitute a major source of water pollution. The industrial effluents were discharged into open drains which finally joins the rivers (Kumari *et al*, 2006).

Wastewater discharge of industries are major issues of water pollution, contributing to oxygen demand and nutrient loading of the water bodies promoting toxic destabilized aquatic ecosystem (Morrison *et al,* 2001; DWAF and WRC, 1995). High or low pH values in a river have been reported to affect aquatic life which alters the toxicity of other pollutant in one form or the other (DWAF, 1996c). A low pH value in a river impairs recreational uses of water and affects aquatic life. A decrease in pH values reduces the solubility of certain essential element such as selenium and increases the solubility of many other elements such as Al, B, Cu, Cd, Hg, Mn and Fe (DWAF, 1996c).

Water quality characteristic of aquatic environment arise from a physical, chemical and biological interactions (Deuzane, 1979; Dee, 1989). Aquatic ecosystem balance get upset by human activities, resulting in pollution which is manifested dramatically as fish kill, offensive taste, odour, colour and unchecked aquatic weeds. The quantity of waste in different phases of a natural aquatic system is reflected by the level of hardness, alkalinity, free CO_2 and other physico-chemical parameters (EPA, 1976). Heavy metals such as lead, cadmium, mercury, nickel, zinc, aluminium, arsenic, copper and iron are mentioned as environmental pollutants, which may cause severe poisoning conditions (Derek, 1999; Dias *et al*., 2002; Ballantyne *et al*., 1999).

The availability of good quality water is an indispensable feature for preventing diseases and improving quality of life (Oluduro and Adewoye, 2007). Natural water contains some types of impurities whose nature and amount vary with source of water for example metals are introduced into aquatic system through, weathering of rocks and leaching of soils, dissolution of aerosol particles from the atmosphere and from several human activities,

including mining, processing and the use of metal based materials (Ipinmoroti and Oshodi, 1993; Adeyeye, 1994; Asaolu *et al.,* 1997). Metals after entering the water may be taken up by fauna and flora and eventually, accumulated in marine organisms that are consumed by human beings (Asaolu *et al.,* 1998).

Yamuna is one of the important river of India. It is used as a source of drinking water and irrigation but due to rapid industrialization, deforestation and urbanization there is a large discharge of industrial waste and sewage into the river which is not safe for human beings, animals, fishes and birds. Now a day direct use of river water for drinking purpose bears significant problem (Barik and Patel, 2004). Fertilizer industry is one of the major water consuming industries responsible for water and soil pollution of considerable magnitude (Sunderamoorthy *et al,* 2001). Most of the waste water is being discharged into surrounding water bodies which disturb the ecological balance and deteriorate water quality (Singh *et al,* 2006). Most of the rivers and fresh water streams are seriously polluted by industrial wastes which come from different industries such as those of petro-chemicals, fertilizers, oil refineries, pulp, paper, textiles, sugar mills, steel, tanneries, distilleries, drugs and pharmaceuticals, fibres, rubber, plastics etc.The textile industries produce effluents that contain several types of chemicals such as dispersants, levelling agents, acids, alkalis, carriers and various dyes (Cooper,1995).

Many farmers use the effluents of factories for irrigation purpose. These effluents contain many harmful materials. In recent years, the industrial effluents are used after treatment for irrigation (Om *et al,* 1994).

New technologies are being proposed to access the treatment of waste water. Algae form one of the components in new technology for waste water treatment. Algal bioremediation has been well studied over the past 40 years by Ryther (1972), Kuyucak (1988), Romero-Gonzalez (2001) and Wang (2011).Considerable research efforts have been devoted to the development of algal biosorbents to remediate pollutants, particularly heavy metals (Hubbe,2011).

Algae are important bioremediation agents, and are already being used in wastewater treatment. The potential for algae in wastewater remediation is however much wider in scope than its current role (Volesky, 1990; Wase and Forster, 1997). Blue green algae (Cyanobacteria) are considered as a most primitive photosynthetic prokaryotes which are supposed to have appeared on this planet during the Pre-Cambrian period (Ash and Jenkins, 2006). Possibly, these are first photosynthetic microorganisms which persisted over a period

of 2-3 billion years, performing an important role in evolution of higher forms. Cyanobacteria are a unique assemblage of organisms which occupy a vast array of habitats (Abd Allah, 2006 and Haande *et al*, 2010).

Cyanobacteria are very susceptible to sudden physical and chemical alterations of light, salinity, temperature and nutrient composition (Boomiathan, 2005 and Semyalo, 2009). Cyanobacteria show immense potential in waste water and industrial effluents treatment, bioremediation of aquatic and terrestrial habitats, chemical industries, biofertilizers, food, feed, fuel etc (Cairns and Dickson, 1971).

Spirulina sp. is a cyanobacterium that grows rapidly (Henrikson, 1989) contains detectable level of mercury and lead (Slotton *et al*., 1989) when grown under the contaminated condition this implies that it can take up toxic metals from the environment. Cyanobacterial species such as *Oscillatoria salina, Plectonema terebrans, Aphanocapsa* sp. and *Synechococcus* sp., developed as mats in aquatic environments, have been successfully used in bioremediation of oil spills in different parts of the world (Raghukumar *et al.,* 2001; Radwan and Al-Hasan, 2001; Cohen, 2002).

Most of the biological treatment technologies involve the use of bacteria, but microalgae have already been applied for effluent treatment, either as single species, as is the case of *Chlorella, Scenedesmus* or *Arthrospira* (Lee, 2001; Lima, 2004; Mulbry, 2001 and Voltolina, 2005) or as mixed cultures/consortia (Mulbry, 2001; Ogbonna, 2000 and Tarlan, 2002) to treat and remove nitrogen, phosphorus and chemical oxygen demand, from different types of effluents. These organisms are also able to remove and incorporate heavy metals, such as lead (Aksu, 1991), Cadmium, nickel or mercury (Chen, 1998 and Travieso1999) present in effluents and their use could be potentially more widespread. Among the several microalgae used to treat effluents *Chlorella* is found to grow in a mixotrophic environment (Karlander, 1996). Investigations conducted by several researchers demonstrated that *Spirogyra* sp. is capable of accumulating heavy metals like Copper, Chromium, Zinc and Fluoride (Bisnhnoi *et al*, 2005).

Few species of marine algae such as *Ascophyllum* and *Sargassum* are effective in the biosorption of pollutants (Volesky and Fourest, 1996; Yu et al, 1999). The major advantage of this is that concentrations of heavy metals in the polluted environment are reduced to a very low level. In the past 20 years the use of immobilized enzymes or cell components for the production of a series of metabolites has become a branch of biotechnology of rapidly

growing importance. The use of immobilized algae in the removal of heavy metal is efficient and offers significant advantages in bioreactors (Hameed and Ebrahim, 2007).

Huge load of wastes from industries, domestic sewage and agriculture practices find their way into rivers, pond resulting in large scale deterioration of water quality leading to the availability of potable water. There is an urgent need to screen and develop efficient alga for the bioremediation of waste water. Keeping this fact the research work on "Potential of Algae in Bioremediation of Waste Water" will be taken.

REVIEW OF LITERATURE

Bioremediation is a newer approach directed towards the treatment of decontamination. Bioremediation primarily deals with the strategies that can employ to clean up the contaminants biologically. Removal and recovery of heavy metals from wastewater is important for environmental protection and human health. Bioaccumulation process is known as an active mode of metal accumulation by living cells which depends on the metabolic activity of the cell **(Volesky, 1990; Wase and Foster, 1997)**.

Microalgae are not unique in their bioremoval capabilities while they offer advantages over other biological materials in some conceptual bioremoval process schemes. Microalgae strains purposefully cultivated and processed for specific bioremoval applications and have the potential to provide significant improvements in dealing with the world-wide problems in metal pollution **(Edward and Benemann, 1993).** It is reported that biosorption of heavy metals by certain types of non-living biomass is a highly cost-effective new alternative for the decontamination of metal-containing effluents **(Kratochvil and Volesky, 1998)**.Biosorption of heavy metals from algae can be effective process for the removal and recovery of heavy metals ions from aqueous solution **(Kaewsarn, 2002).**

Chlorella vulgaris and *Scenedesmus dimorphus* is highly efficient for ammonia and phosphorous removal during biotreatment of secondary effluents from an agro industrial wastewater of a dairy industry and pig farming. These microalgae were isolated from wastewater stabilization pond. Both these microalgae removed phosphorous from the wastewater to the same extent **(Luz Estela Gonzalez, 1997).** Dead dried *Chlorella vulgaris* was studied in terms of its performance in binding divalent copper, cadmium, and lead ions from their aqueous or 50% v/v methanol, ethanol, and acetone solutions. The percentage uptake of cadmium ions exhibited a general decrease with decrease in dielectric constant values, while that of copper and lead ions showed a general decrease with increase in donor numbers **(Al-Qunaibit, 2009)**

Algae have received increasing attention for heavy metal removal and recovery due to their good performance, low cost and large available quantities **(Wang and Chen 2008)**. *S. incrassatulus* was also able to remove all the tested metals to some extent (25-78%), but bivalent metals were not removed as efficiently as reported in batch cultures, probably due to the high pH values there recorded. Chromium (VI) was more efficiently removed in continuous cultures than in batch culture, because the uptake of chromate could be favoured

by actively growing algae **(Peña-Castro *et al* 2004)**. Micro-algae can be used for tertiary treatment of wastewater due to their capacity to assimilate nutrients. The pH increase which is mediated by the growing algae also includes phosphorous precipitation and ammonia stripping to the air, and may in addition act disinfecting on the wastewater **(Karin Larsdotter, 2006)**.

Algae have been proven efficient biological vectors for heavy metal uptake. Biosorption potential of two strains *Spirogyra sp.* and *Spirulina sp*. has been studied under different initial metal concentrations **(Mane and Bhosle 2012)**. The use of live and dead *Spirulina* sp. for sorption of metals like Cr3þ, Ni2þ, Cu2þ and Cr6þ in form of Cr_2O_2 *Spirulina* sp. treated with different metal ions have been employed to understand the sorption mechanism. It is hoped that live *Spirulina* sp.will be a strong candidate for management of industrial wastewater **(Doshi *et al* 2007)**.

Huijuan Meng (2011) reported the biodegradation rates of linear alkyl benzene suffocate LAS by Spirulina *platensis* increased with Zn (II) and reached the maximum when Zn (II) was 4 mg/L. The joint toxicity test showed that the combined effect of LAS and Zn (II) was Synergistic. LAS can enhance the biosorption of Zn (II), and reciprocally, Zn (II) can enhance LAS biodegradation. **Bindiya *et al* (2012)** observed the Bioaccumulation of Cadmium in Blue Green Algae *Spirulina* (Arthrospira) *Indica.*

Deng *et al* (2007) reported that *Cladophora fascicularis* green alga is highly efficient for the biosorption of copper (II) from aqueous solution. Biosorption is the effective method for the removal of heavy metal ions from wastewater. Results are presented showing the sorption of Pb (II) from solutions by biomass of commonly available, filamentous green algae *Spirogyra* sp **(Gupta and Rastogi 2008). Khalaf (2008)** observed the Biosorption of reactive dye from textile wastewater by non-viable biomass of *Aspergillus niger* and *Spirogyra* sp.

Monteiro *et al* (2009) observed that strains of the *Scenedesmus obliquus* microalga tested have proven effective in removing a toxic heavy metal from aqueous solutions, hence supporting their choice for bioremediation strategies of industrial effluents. It was proven, for the first time, that such a wild microalgae can uptake and adsorb Zn very efficiently, which unfolds a particularly good potential for bioremediation. Its performance is far better than similar (reference) species, especially near neutrality, and even following heat-treatment **(Monteiro, 2011).**

Kaushik *et al* (2008) reports on chromium (VI) tolerance of two cyanobacterial strains *Nostoc linckia* and *Nostoc spongiaeforme* isolated from salt affected soils using uni-algal and bi-algal systems. It was observed that the effectiveness of cyanobacterial concern because they colour and diminish the quality of water bodies into which they are released. The effectiveness of *Oscillatoria* was employed for the bioremediation of textile effluents **(Abraham and Nanda, 2010)**.

Prado *et al* (2010) observed the rate of biosorption of cadmium and copper ions by non-living biomass of the brown macroalga *Sargassum sinicola* under saline conditions. They see that presence of salt did not significantly affect the rate of biosorption and there is an antagonistic effect on biosorption when both these metals are present in the solution.

Among the several microalgae used to treat effluents *Chlorella* is often found from the various types of waste water for the treatment of the water **(Karlander and Krauss, 1996). Raposo *et al* (2010)** analyzed the capacity of *Chlorella vulgaris* and the autochthonous flora of the effluents to remove some of the compounds present in the effluents. **Cecal *et al* (2012)** deals with a study of the biosorption of UO_2^{2+} ions on two green algae: *Chlorella vulgaris* and *Dunaliella salina*. By kinetic investigations it was found that the biosorption process was greater for *Chlorella vulgaris* than for *Dunaliella salina.*

Kannan (2011) seen the detoxification capacity of a variety of microbes especially cyanobacteria. They collected the effluents from tannery industry and added to the cyanobacterial growth medium in various proportions. The photosynthetic pigments and nitrogen status of *Anabena flos-aquae* were analysed before and after the treatment with effluents. It showed that *Anabena flos-aquae* can serve as the potential bioremedial organism for industrial pollution.

Biodegradation and biosorption capacity of some potential cyanobacterial species: *Oscillatoria* sp., *Synechococcus* sp., *Nodularia* sp., *Nostoc* sp. and *Cyanothece* sp. dominated the effluents and mixed cultures showed varying sensitivity. Contaminants were removed by all the species either as individuals or in mixtures **(Dubey *et al,* 2011)**. **Lee and Chang (2011)** observed the biosorption capacity from aqueous solutions of the green algae species, *Spirogyra* and *Cladophora*, for lead (Pb (II)) and copper (Cu (II)). In comparing the analysis of the Langmuir and Freundlich isotherm models, the adsorption of Pb (II) and Cu (II) by these two types of biosorbents showed a better fit with the Langmuir isotherm model.

Miranda *et al* (2012) observed two species of cyanobacteria, *Oscillatoria laete-virens* (Crouan & Crouan) Gomont and *Oscillatoria trichoides* Szafer which were isolated from a polluted environment and it is studied for their Cr^{6+} removal efficiency from aqueous solutions.

Bioaccumulation is the effective method for removal of heavy metal ions from wastewaters. Bioremediation, the use of algal to extract, sequester and or detoxify heavy metals and other pollutants. They use filamentous alga of *Pithophora* sp. for the removal of cadmium, chromium and lead from industrial wastewater **(Brahmbhatt *et al* 2012).** Mercury is higher in *Dunaliella* alga as compared to those of cadmium and plumb. This is vivid that *Dunaliella* is highly tolerant to the ascending concentration of heavy metals and their absorption in aquatic media. This approves the usage of *Dunaliella* as useful equipment for the elimination of heavy metals environment **(Imani *et al* 2011)**.

Gao and Yan (2012) observed the Response of *Chara globularis and Hydrodictyon reticulatum* to lead pollution: their survival, bioaccumulation, and defense they observed that *H. reticulatum* exhibited higher tolerance to Pb pollution than *C. globularis.* Some workers determine the feasibility of using algae growing in wastewater lagoons to absorb residual heavy metals for subsequent complete removal by algae-intermittent sand filtration system and they found that this technique is very helpful in removal of certain heavy metals from wastewater **(Daniel *et al,* 1979).**

The major problem in utilization of microorganisms in any industrial or waste water treatment is harvesting of the biomass. This is solved by the strategy of immobilization. Immobilization technique is essential not only in waste water treatment but also in various industries **(Prakasham and Ramakrishna 1998).**

One of the main interests for microalgae in biotechnology is focussed on their use for heavy metals removal from effluents and waste water **(Mallick, 2002).**Some research has been done dealing with the immobilization of microalgae for different purposes: morphology studies, the production of fine chemicals, energy production, wastewater treatment etc. Immobilization strains of microalgae is been used for sewage treatment. Efficiency of depuration was highest when a fluidized bed and *Chlorella vulgaris* were used **(Travieso, 1992).**

Use of immobilized algae in wastewater treatment and heavy metal removal processes efficient and offer significant advantages in bioreactors **(Hameed and Ebrahim, 2007). Tam *et al,* 1994** used *chlorella vulgaris* cells immobilized in alginate beads for removing N and P

from wastewater and they achieved significant reductions in wastewater ammonia and phosphate. *Spirulina platensis,* a cyanobacterium of economic important was studied for the tolerance to cadmium.

The Biosorption studies showed that the algae had a great potential for adsorbing the heavy metal on to the cell. The immobilized cell of *Spirulina platensis* was able to be more effective in absorbing the metal to the cell **(Murugesan *et al*, 2008).**

The process of biosorption of trivalent chromium (Cr^{3+}) by live culture of *Spirulina platensis* and the sorption potential by the dried biomass, in both free and immobilized states have been investigated for simulated chrome liquor in the concentration range of 100–4500 ppm. Both live and dried biomass were very good biosorbents as they could remove high amounts of chromium from tannery wastewater **(Shashirekha *et al*, 2008)**

OBJECTIVES

1. Collection of waste-water samples from different sites of Agra.

2. To study the waste- water samples collected from different sites with respect to –

2. a. Physico-chemical aspects

i. Colour and pH
ii. Acidity and Alkalinity
iii. Hardness
iv. TSS (Total Suspended Solids), TDS (Total Dissolved Solids) and TS (Total Solids)
v. DO (Dissolved Oxygen), BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand)
vi. Organic Carbon and Ammonical N_2

2. b. Identification of Algae

3. Collection, Identification, Isolation and Culture of specific algae *(Spirulina* sps., *Hydrodictyon* sps., *Spirogyra* sps., *Chlorella* sps)

4. Assessment of the specific algal isolates (*Spirulina* sps., *Hydrodictyon* sps., *Spirogyra* sps., *Chlorella* sps., its mixed culture etc) in the bioremediation of the waste water samples and screening the most efficient algal isolate.

METHODOLOGY

1. Collection of waste-water samples from different sites of Agra.

Water samples will be collected from different sites of Agra in a 2 litre bottle which will previously washed with 10% HNO_3 for 48 hr, labelled these bottles and few drops of HNO_3 will add to prevent loss of metals.

2. To study the waste-water samples collected from different sites with respect to –

2. a. Physico-chemical aspects

The physico-chemical parameters of collected waste water samples will be determined before and after treatment by following the Standard Method Examination of Water and Waste Water given in "Environment and Pollution" of Ambast (1990) and APHA (1998). The data will also be statistically analysed by taking the value of standard error (Chandel, 1999).

i) Colour and pH

- **Colour**- the colour intensity of water will be observed from naked eyes.

- **pH**- the pH will be measured by the digital pH meter. Calibration of the pH meter will be accomplished by pH electrode submerged in a pH 7 buffer solution. pH measurement will be made by placing pH electrode tip 6-8cm into the sample and then recording the meter reading after the stabilization.

ii) Acidity and Alkalinity

- **Acidity-**

Reagents:-

  - **Methyl Orange**
  - **NaOH**

- **Phelophthalein**

Procedure-

100 ml of water sample will be taken in a flask in which 2 drops of methyl orange is added; it turned pink and then titrate it with 0.05N NaOH solution. At the end point the pink colour change to light yellow. Now added 3-4 drops of phenolphthalein and continued to titrate it until the end point from yellow to pink is changed. Following formula is used to calculate the acidity-

$$\text{Acidity (mg/l) as } CaCO_3 = \frac{\text{NaOH total titration vol. in ml} \times 0.05N \times 1000 \times 50}{\text{ml of sample taken}}$$

- **Alkalinity-**

Reagents:-

- **Phenolphthalein indicator** – 0.5gm phenolphthalein indicator will be dissolved in 50ml of 95% ethyl alcohol and 50ml of distilled water 0.05N NaOH was added drop by drop until colour becomes just like pink.

- **Indicator methyl orange**- 0.5gm methyl orange is dissolved in 100ml distilled water.

- **0.1N HCl**- it was prepared by taking 8.34ml HCl and diluted it in 1litre distilled water.

Procedure-

Take 100ml water sample and add 2 drops of phenolphthalein indicator. Solution turns pink and titrated with the dil. HCl. The end point come with sharp disappear of pink colour volume of dil. HCl will be noticed. Now in same flask 2-3 drops of methyl orange will be added and the colour of solution turns yellow. Further titration continued and a new end point reached when a solution in the flask is just turns to pink. Total alkalinity will be calculated by following formula-

$$\text{Alkalinity} = \frac{\text{Total HCl} \times 0.1\text{N HCl} \times 1000 \times 50}{\text{ml of the sample}}$$

iii) Hardness- the hardness of water sample will be measured by EDTA Titrimetric Method (Ambast, 1990).

Reagents:-

- **Buffer solution (pH=10)**

  16.9gm ammonium chloride will be dissolved in 143ml of concentrate ammonium hydroxide.

  1.179gm di sodium EDTA (Ethylene ditetra Acetic Acid) and 0.78gm $MgSO_4.7H_2O$ will be dissolved in 50ml distilled water. Both prepared solution is mixed and volume it up to 250ml by adding distilled water.

- **Erichrome Black T indicator (EBT)** – 0.5gm dye will be dissolved in 100ml nitrotriethanol.

- **EDTA Titrant (0.01M)** - 3.723 gm di Sodium salt of EDTA will be dissolved in distilled water and raised a volume of it upto 1 litre with distilled water.

Procedure-

50ml of sample will be taken in a conical flask. 1-2 ml of buffer solution and 1-2 drops of EBT indicator will be added into the flask. The solution turns wine red. The sample will be titrated against standard EDTA Titrant. The sample will be titrated up to the end point till the colour turn from wine red to blue and notice the titrant reading. The following formula will be used-

$$\text{Hardness (mg/l)} = \frac{\text{EDTA used (ml)} \times 1000}{\text{ml of sample.}}$$

iv) TSS (Total Suspended Solids) TDS (Total Dissolved Solids) and TS (Total Solids)

- **Total suspended solids (TSS)**

For the measurement of TSS a known volume of sample will be titrated through oven dried pre-weighted filter paper and the residue containing filter paper was oven dried at 100ºC and again weighted.TSS of the sample will be calculated by following formula-

TSS (mg/l) = initial weight of filter paper- final weight of filter paper

- **Total dissolved solids (TDS)** –

Water sample will be taken and then filtered it to remove suspended particles. 250ml of clear filtrate will be evaporated in an oven at 100°C in porcelain disc. Measurement will be observed by-

$$TDS\ (mg/l) = \frac{W2-W1\times1000}{V}$$

Where,

W1= weight of empty disc

W2= weight of oven dried disc

V= volume of sample taken (ml)

- **Total Solids (TS)**

Total solids include both suspended and dissolved solids. It is calculated by using the formula-

$$TS\ (mg/l) = TSS+TDS$$

v) Dissolved Oxygen (DO), Biological Oxygen Demand(BOD) and Chemical Oxygen Demand(COD)-

- **Dissolved Oxygen (DO)**

Reagents:-

- **Conc. H_2SO_4**

- **Manganous sulphate solution-** it will be prepared by dissolving 364gm $MnSO_4$ in distilled water and dilute to 1litre.

- **Alkali iodide azide solution-** it will be prepared by dissolving 700gm KOH and 150 gm KI in 1 litre of distilled water. Then 10gm sodium azide (NaH_3) will be dissolved in 40ml distilled water and added to above solution.

- **Starch solution-** prepared by forming an emulsion of 0.5gm starch in a beaker with a small quantity of distilled water. This emulsion will be poured in 100ml boiled distilled water and solution will be boiled 5-6 minute and settled overnight. Supernatant was taken as starch indicator.

- **Sodium thiosulphate solution (0.1N)** - 24.82gm NaS_2O_3 will be dissolved in boiled distilled water and on cooling diluted it in 1litre distilled water. 0.025N NaS_2O_3 will be prepared by diluting 250ml NaS_2O_3 stock to 1000ml distilled water.

Procedure-

Water sample will be collected without bubbling in the 250ml glass bottle. 2ml each of mangnous sulphate and alkali iodide azide solution will be added right at the bottom of the bottle with separate pipettes. The bottle will shake at least six times. The brown precipitate formed allowed to settle, 2ml concentrate sulphuric acid will be added and shaken the bottle to dissolve the brown precipitate. 50ml

of solution is taken in a flask and then titrate it with sodium thiosulphate solution (taken in burettes) till the colour change to pale straw. 2 drops of starch solution is added to the above flask. This changed the colour of the content from pale to blue solution that is titrated again with thiosulphate solution till the blue color disappears. The total amount of sodium thiosulphate will be observed and dissolved oxygen content in water (mg/l) is calculated by following formula-

$$\underline{DO\ (mg/l) = (8^* \times 1000 \times N) \times v}$$
$$V$$

Where,

V= volume of the sample taken (ml)

v= volume of the titrant used

N= normality of the titrant

8*= it is the constant since 1.0ml of 0.025 sodium thiosulphate solution is equivalent to 0.2mg of oxygen.

- **Biological Oxygen Demand(BOD)-**

Reagents-

- **Phosphate buffer(pH 7.2)-** 8.5 gm KH_2PO_4, 21.75gm K_2HPO_4, 33.4gm $Na_2HPO_4.7H_20$ and 1.7gm NH_4Cl dissolved in 1litre of distilled water.

- **$MgSO_4$ solution-** 2.25gm $MgSO_4.7H_2O$ will be dissolved in 100ml distilled water.
- **$CaCl_2$ solution-** 2.75gm $CaCl_2$ will be dissolved in 100ml distilled water.
- **$FeCl_3$ solution**- 0.2gm $FeCl_3.6H_2O$ will be dissolved in 1litre distilled water.
- **Sodium Sulphite Solution (0.025N) -** 1.575gm $NaSO_3$ will be dissolved in 1 litre of distilled water.

Procedure-

The dilution water will be prepared by adding 1ml of each phosphate buffer solution, magnesium sulphate solution, calcium chloride solution, ferric chloride solution to 1 litre distilled water. 2ml water sample will be added and aerated. The DO of undiluted sample will be determined which designated as DO_0. The desired percentage mixture was prepared by adding sample in dilution water. One bottle will be filled with the mixture and designated as DO_1 and the other one with dilution water (blank) designated as DO_2. Both bottles will be incubated at 20°C for 5 days and after incubation, the DO will be determined. The BOD will be obtained by the following formula-

$$BOD\ (mg/l) = [\{(DO_2\text{-}DO_1) \times 100\}\ (DO_2\text{-}DO_0)\}]$$

- **Chemical Oxygen Demand(COD)**

Reagents-

- **0.1M Potassium dichromate solution-** 3.676gm $K_2Cr_2O_7$ will be dissolved in 1 litre distilled water.
- **Sodium thiosulphate (0.1M) -** 15.811gm NaS_2O_3 will be dissolved in 2litre of distilled water.
- **Sulphuric acid (2M) -** 10.8ml of concentrate H_2SO_4 will be dissolved in 100ml distilled water.
- **10% of Potassium iodide solution-** 10gm KI will be dissolved in 100ml distilled water
- **1% Starch solution-** 1gm starch will be dissolved in 100ml distilled water.

Procedure-

50ml of water sample will be taken in triplicates of 100ml flask and triplicates of blank will also prepare. 5ml of K_2CrO_7 solution will be added to each 6 flasks. Then kept these flask at 100ºC in water bath for 1hr. the samples will be allowed to cool for 10minutes and then 5ml of KI will be added then add 10ml of H_2SO_4 in each flask. Content of each flask will be titrated in 0.1M $Na_2S_2O_3$ till the appearance of pale yellow color. 1ml of starch solution is added due to which the solution turns

pale yellow to blue and titrated it again until the blue colour disappear completely. COD will be calculated by applying the formula-

$$\text{COD of the sample (mg/l)} = \frac{8\times C\times (B-A)}{S}$$

Where,

C= concentration of the titrant (mM/l)

A= Volume of the titrant used for blank (ml)

B= Volume of the titrant used for sample (ml)

S= Volume of the water sample taken

vi) Organic Carbon and Ammonical N_2

- **Organic Carbon**

Reagents-

- **Sulphuric acid**
- **0.1N Iodine solution**
- **Potassium sulphate**
- **$CuSO_4$**
- **0.1N Sodium thiosulphate**

Procedure-

100ml water sample will be taken in a round bottom flask (Kjeldahl's Flask). 30ml of concentrate H_2SO_4 will be added. Through rubber stopper a thistle funnel will be inserted into Kjeldahl flask, which dipped into sulphuric acid and water mixture. The side tube of the kjeldahl flask will be connected to two flasks arranged in series containing 75ml of 0.1N iodide solution and the flasks will be connected to suction pump. Before inserting the thistle funnel, 4gm of potassium sulphate and 5gm of $CuSO_4$ will be added to the mixture of sulphuric acid and water sample. The kjeldahl flask will be heated with the help of burner till the clear blue colour obtained. The iodine present in flask will be titrated against 0.1N sodium thiocyanate and the organic carbon will be estimated by using the formula-

1ml of 0.1N iodine used=0.003 gm of organic carbon

- **Ammonical N_2**

In water the nitrogen content will be estimated by Kjeldahl method (APHA, 1998).

Reagents:-

- **Sodium hydroxide**
- **0.04N H_2SO_4**
- **Phenolphthalein indicator**

Procedure-

50 ml of water sample will be taken in kjeldahl flask and neutralized it to pH 7. 10ml of concentrate H_2SO_4, 6.4gm K_2O_4 and 1.2ml $HgSO_4$ solution will be added to flask. Few glass beads also added into the flask to prevent bumping. All the material will be mixed and heated under a hood until white fumes will be observed. The material will be digested until the turbid samples will be turned into straw colour. After digestion, 300ml of distilled water and 50ml of NaOH solution will be added into the flask. The flask then connected to the distillation unit. One end of the distillation unit connected to Kjeldahl flask and another end to distillate containing 50ml of 0.04N H_2SO_4 solution. Again kjeldahl flask is heated for half an hour. A blank reagent will also carried using all the steps of procedures. The nitrogen will be estimated by titration method, using phenolphthalein as an indicator. The nitrogen content present in the sample will be calculated by using the formula-

$$\text{Nitrogen (ml/l)} = \frac{\text{A-B} \times 280}{\text{ml of sample used}}$$

Where,

A= volume of the titrant used for sample

B= volume of titrant used for blank

2.b. Identification of Algae

Identified the algae present in these water sample collected from different sites of Agra.

3. Collection, Identification, Isolation and Culture of specific algae *(Spirulina* sps., *Hydrodictyon* sps., *Spirogyra* sps., *Chlorella* sps)

Different types of algae will be subjected for the bioremediation of water.Out of these, few are collected locally (*Hydrodictyon* sps.) from this water samples and identifies on the basis of their morphological characteristics. Species of *Spirulina* will be previously identified in *Spirulina* Laboratory, Department of Botany, D.E.I, Dayalbagh, Agra while remaining will be procured from IARI. Then we will culture these algal samples in different media. Some will be cultured directly in water. While some algae will be cultured in prescribed medium such as CFTRI Medium and BG-11 Medium.

i) CFTRI Medium

Chemicals	**g/l**
$NaHCO_3$	4.5
K_2HPO_4	0.5
$NaNO_3$	1.5
K_2SO_4	1.0
NaCl	1.0
$MgSO_4.7H_2O$	0.2
$CaCl_2$	0.04
$FeSO_4$	0.01
Water	1 litre

i) BG-11 Medium

Chemicals	**gm/l**
$NaNO_3$	1.5 g
K_2HPO_4	0.04 g
$MgSO_4 \cdot 7H_2O$	0.075 g
$CaCl_2 \cdot 2H_2O$	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium salt)	0.001 g
$NaCO_3$	0.02 g
Trace metal mix A5	1.0 ml
Distilled water	1.0 L

pH should be maintain 7.1 after sterilization

Trace metal mix A5:

Chemicals	**gms/l**
H_3BO_3	2.86 g
$MnCl_2 \cdot 4H_2O$	1.81 g
$ZnSO_4 \cdot 7H_2O$	0.222 g
$NaMoO_4 \cdot 2H_2O$	0.39 g
$CuSO_4 \cdot 5H_2O$	0.079 g
$Co(NO_3)_2 \cdot 6H_2O$	49.4 mg

4. **Assessment of the specific algal isolates (*Spirulina* sps. *Hydrodictyon* sps. *Spirogyra* sps., *Chlorella* sps., its mixed culture etc) in the bioremediation of the waste water samples and screening the most efficient algal isolate.**

300ml of different water samples will be inoculated with 30ml algal isolates of particular density in a flask and kept it under illumination at 30°C then observed it after the interval of 10days upto 30days under aerobic condition. For first 48 hr of incubation, the flask will be kept in a shaker at 100rpm for the purpose of uniform mixing of algae and effluents. Then periodically monitoring of the samples will be done for investing the physiochemical characteristics and biodegradability of the effluents. On the basis of Physico-chemical analysis the most efficient algal isolate will be screened on the basis of their reduction efficiency in BOD, DO and COD.

SIGNIFICANCE

Algal bioremediation is considered as an efficient and environmentally safe technology for inexpensive decontamination of polluted systems. It is widely used for heavy metal removal from waste water. The objective of the proposed study is to isolate the most efficient naturally occurring algal species with high bioremediation capabilities of water bodies in Agra.

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